Pollen morphology of *Cayaponia* Silva Manso (Cucurbitaceae) Brazilian species

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Abstract

The pollen morphology of 29 species of *Cayaponia* (Cucurbitaceae) native in Brazil and one native species in Mexico was studied. The pollen grains were prepared using the acetolysis method for light microscope observations. In sequence, the acetolysed pollen grains were dehydrated and gold sputtered for surfaces analysis using scanning electron microscopy. Five pollen types in accordance to pollen grain size and number of apertures and in addition 9 sub-types related to spine indices could be established. Species are characterized by the spine configurations. A pollen key was elaborated for species identification based on light microscope observations only. In some cases several species were forming a single pollen sub-type.

Key words: pollen morphology, *Cayaponia*, Cucurbitaceae.

Introduction

The family Cucurbitaceae consists of circa 120 genera and 825 species that occur all over the tropical and subtropical regions of the world (3). The genus *Cayaponia* (circa 60 species in America) is represented by circa 44 native species in Brazil. They can be found in several types of vegetation, frequently in forests.

Pollen morphology was studied at the moment in relation to only some species, most of them from Central America (8). Campos (4) and Salgado-Labouriau (9) analyzed *C. espelina* of the Brazilian savanna ("cerrado"). The present study includes 29 of the native species occurring in Brazil and 1 native species of Mexico.

Materials and Methods

The botanical material was obtained from the following herbaria: Museu Nacional do Janeiro (R), Jardim Botânico do Rio de Janeiro (RB), Universidade Federal de Goiás (UFG), FEEMA – Centro de Botânica do Rio de Janeiro (GUA), Universidade de Brasília (UB), Universidade Santa Úrsula (RUSU), Royal Botanical Garden (K), Instituto de Botânica de São Paulo (SP), Herbário Bradeanum (HB), Reserva Ecológica do IBGE (IBGE), Instituto Nacional de Pesquisas da Amazônia (INPA), Herbário da Universidade Federal de Juiz de Fora (CESJ), Herbário Barbosa Rodrigues (HBR), Universidade de São Paulo (SPF) e Herbário do Centro de Pesquisas do Cacau (CEPEC).

Fertile anthers were processed according to the standard methodology of acetolysis (5). The measures of the pollen grains were taken soon after acetolysis, in a maximum term of a week. The arithmetic average and the standard deviation of the average of the polar and equatorial axes were based on measurements of 25 pollen grains randomly. The polar axis/equatorial axis (P/E) proportion was used to define the form of isopolar and radially symmetric pollen grains. Other details of pollen morphology was based on the measurements of 10 pollen grains. The width and the height of the pores were measured in accordance to the equatorial and polar axis respectively and the thickness of the sexine and nexine in the median area between two pores.

The width of the spine apex was measured 1 µm away its apex. The spine index, which defines the spine configuration, is the proportion between the height and width of the spine at its basis. Spines with indices lower than 1.5 are named short spines, when between 1.5 and 2.0 medium spines and when more than 2.0 long spines.

The acetolysed pollen grains were dehydrated and gold sputtered for surfaces analysis using scanning electron microscopy (ZEISS DSM 940 microscope).

The terminology used follows the glossaries of Barth & Melhem (2) and Punt & al. (7).

Results

Pollen grains of *Cayaponia* are of large and gigantae sizes (50 - >200 µm of diameter), spheroidal or suboblate, 3 to 8-zonoporate or pantoporate. The pori are operculate presenting annulus. The sexine presents spines of different sizes and numerous densely spaced pila between; in some species capita of the pila are fusioned (Figs. 16, 17, 19 and 20). The nexine 1 is thicker than the nexine 2. Using light and scanning electron microscopy, it was possible to group the studied specimens into 5 pollen types based upon the size of the pollen grains, the distribution of the pori and the feature of the spines.

The results can be summarized in the following key, based on light microscopy observations:

1. grains of large size (50µm – 100 µm)

1.1. grains 3- to 6-zonoporate
1.1.1. spine indices > 2.0
1.1.1.1. apex variable (pointed and segmented pointed).....*Cayaponia nitida* (Jardim 229) (4-porate) (Fig. 13)

1.1.2. spines indices 1.5 - 2.0
1.1.2.1. pointed apex.....*C. alfrediana* (3 to 6-porate), *C. palmata* (Glaziou 12023) (3 to 4-porate), *C. palmata* (Pereira & Pabst 6896) (3-porate) (Figs. 1, 2), *C. pilosa* (3 to 4-porate)
1.1.2.2. rounded apex.....*C. weddellii* (Silva et al. 1860) (4 to 5- porate)
1.1.2.3. variable apex (faceted and irregular).....*C. nitida* (Silva & Emmerich 3489) (4-porate) (Fig. 12)

1.1.3. spine indices < 1.5.
1.1.3.1. pointed apex.....*C. trifoliolata* (Gomes- Klein & Pereira 484) (4 to 5-porate)
1.1.3.2. faceted apex.....*C. longifolia* (3 to 6- porates) (Fig. 11)

1.2. pantoporated grains (4- to 6-porate), spine index < 1.5; variable apex (pointed and faceted).....*Cayaponia martiana* (Figs. 3, 4, 14)

2. grains of very large size (100 µm – 200 µm)

2.1. grains 3- to 6-zonoporate
2.1.1. spine indices > 2.0
2.1.1.1. pointed apex.....*C. trifoliolata* (Kuhlmann 2751) (4 to 6-porate)
2.1.1.2. faceted apex.....*C. citrullifolia* (Cesar 340) (3-porates), *C. citrullifolia* (Gomes-Klein & Cid 3419) (3-porate), *C. citrullifolia* (Falcão 5120) (3 to 4-porate) (Fig. 17), *C. bonariensis* (3 to 4- porate), *C. ferruginea* (4 to 5-porate)

2.1.2. spine indices 1.5 - 2.0
2.1.2.1. pointed apex.....*C. cabocla* (3-porate) (Figs. 5, 6), *C. villosissima* (4-porate), *C. botryocarpa* (SP 225525) (5- to 6-porates), *C. alarichii* (3-porate)
2.1.2.2. faceted apex.....*C. diversifolia* (RB 88371) (4- to 6-porate), *C. trilobata* (4- to 6-porate), *C. gracilliama* (4- to 5-porate)
2.1.2.3. rounded apex.....*C. rigida* (4-porate), *Cayaponia* sp. (Anderson 9147) (6 to 7-porate), *C. rugosa* (3 to 4-porates), *C. tubulosa* (Kuhlmann 859) (5 to 6-porate) (Fig. 16)

2.1.3. spine index < 1.5
2.1.3.1. pointed apex.....*C. membranacea* (3-porate) (Fig. 15), *C. caulobotrys* (4- to 5-porate), *C. diversifolia* (Santos 70) (4- to 6-porate), *C. duckei* (4- to 6-porate), *C. botryocarpa* (INPA 147266) (6-porate)
2.1.3.2. faceted apex.....*Cayaponia* sp. (Liesner 6801) (4- to 5- porate)
2.1.3.3. rounded apex.....*C. petiolulata* (4 to 6-porate), *C. tubulosa* (Coelho & Rosas 1913) (5- to 6-porate)

2.2. pantoporated grains (4- to 8-porate)

2.2.1. spine indices > 2.0
2.2.1.1. pointed apex.....*C. tayuya* (Gomes-Klein 3426) (4- to 6-porate), C. tayuya (Gomes-Klein 3421) (5- to 6-porate), C. tayuya (Gomes-Klein 3422) (7-porate) (Figs. 7, 8, 20)

2.2.2. spine indices 1.5 – 2.0
2.2.2.1. pointed apex.....*C. racemosa* (6- to 7-porate)
2.2.2.2. faceted apex.....*C. espelina* (Krieger 19446) (5- to 6-porate) (Fig. 19), *C. macrocalyx* (6-porate), *C. weddellii* (Cesar 577) (6- to 8- porate)

2.2.3. spine indices < 1.5.
2.2.3.1. faceted apex.....*C. espelina* (Gomes-Klein 2640) (4 to 5-porate) (Fig. 18)
2.2.3.2. rounded apex.....*C. weddellii* (Pirani et al.1712) (4 to 7-porate)

3. grains of gigantae size (200µm), pantoporate; spine index 1.5 – 2.0, faceted apex *Cayaponia fluminensis* (Figs. 9, 10, 21).

Discussion

Cayaponia is a stenopalynous genus.

The monospecific pollen type of *C. fluminensis* is distinguishable from all the others specimens examined, while presenting gigantae sized and pantoporate pollen grains, with medium spines of acute apexes.

Two other pollen types comprise pollen grains of large size, 3- to 6-zonoporate or pantoporate (*C. martiana*). *C. nitida* only presents 4-porate pollen grains; however the examined specimens differ regarding the features of the spines, that are long with acute and acute-segmented apexes in the specimen Jardim 229 and medium sized with conic and irregular apexes in specimen Silva & Emmerich 3489. Variation and superposition of the number of pores occur in pollen grains of *C. alfrediana*, *C. palmata* and *C. pilosa*, presenting medium spines of acute apexes, forming a group of indefinable species. Pollen grains with short spines with faceted apexes are found in *C. longifolia.*

The very large size pollen grains include other two pollen types. They are 3- to 6-zonoporate or pantoporate. Among zonoporate pollen grains it is possible to distinguish the species based on the configuration of the spines: long spines of faceted apexes occur in *C. bonariensis, C. citrullifolia* and *C. ferruginea;* spines of medium size with acute apexes occur in *C. alarichii, C. cabocla, C. botryocarpa (SP 225525)* and *C. villosissima;* spines of medium size with faceted apexes occur in *C. gracilliama* and *C. trilobata*; spines of medium size with rounded apexes occur in *C. rigida* and *Cayaponia* sp. (W.R. Anderson 9147). The species which also presents short spines have variations of acute apexes in *C. botryocarpa* (INPA 147266), *C. caulobotrys, C. duckei* and *C. membranaceae*; of faceted apexes in *Cayaponia* sp. (R. L. Liesner 6801), and rounded apexes in *C. petiolulata*. Variations regarding the feature of the spines also occur among pantoporate pollen grains: long and of acute apexes in *C. tayuya*; mediums and of acute apexes in *C. racemosa* and of faceted apexes in *C. macrocalyx*.

There were divergences in some species when more than one exsicata was examined. *C. trifoliolata*, specimen Gomes-Klein & Pereira 484, it frames in the zonoporate pollen grains group with large size, presenting short spines; specimen Kuhlmann 2751 it frames in the zonoporate pollen grains group of very large size, presenting long spines. The variations found among specimens of the *C. botryocarpa*, *C.* apexes occur in *C. diversifolia* collection RB 88371 and with short spines of acute apexes in specimen Santos 70. Similar fact occur in *C. tubulosa*. The specimen Kuhlmann 859 presents medium spines and the specimen Coelho & Rosas short spines, in both the apexes are rounded. For *C. botryocarpa*, the specimen SP 225525 presents medium spines and the specimen INPA 147266 presents short spines, both with acute apexes and their provenience are from the same collection, however deposited in different herbaria. The pantoporate pollen grains of *C. espelina* present medium spines in the specimen Krieger 19446 and short spines in the specimen Gomes-Klein 2640 and both have faceted apexes.

Differently, the *C. weddellii* (Silva et al. 1860) specimen comprise large zonoporate pollen grains, with medium spines of rounded apexes, while the other two collections present additional variations regarding distribution of the pore, size of the pollen grains, size and form of the spines. Very large size pantoporate pollen grains, with short spines and rounded apexes are observed in specimen Pirani et al. 1712, and with medium spines and faceted apexes in Cesar 577.

The pollen grains of the specimen *C. cabocla* here examined are 3-zonoporate, while Marticorena (6) related larger grains varying from 3-zonoporates to 4-pantoporates.

The morphological characteristics of the pollen grains of the two specimens of *C. espelina* here examined are confirmed by Campos (4) and Salgado-Labouriau (9), having small variations in the referred dimensions.

The 6-7-pantoporate pollen grains of *C. racemosa* here analyzed, differ from the material studied by Roubik & Moreno (8), that presents 10-pantoporate pollen grains, and those by Ayala-Nieto et al. (1) that present 8-10-pantoporate pollen grains. Regarding the feature of the exine, Roubik & Moreno (8) presented a semitectum and Ayala-Nieto et al. (1) presented a tegillum in *diversifolia, C. espelina* and *C. tubulosa* are less accentuaded, maintaining itself included at the same pollen type. The variation among specimens is restricted to the configuration of the spines. The very large zonoporate pollen grains, with medium spines of faceted the areas between the spines. This may be a lack of resolution, since scanning electron microscope observations confirmed the existence of pila and a lack of tectum. Regarding the examined material by Erdtman (5), the differences refer to the feature of the spines, with apexes rounded to faceted in the specimen studied by him.

According to the morphological data that exist nowadays about *Cayaponia*'s pollen intra and interspecific variations, mainly the number and distribution of pores as well as the configuration of the spines and the height of pila (thickness of the sexine) are of significant characteristics. The species that are morphologically defined are: *C. fluminensis, C. martiana, C. citrullifolia, C. palmata* and *C. tayuya*. The species that present morphological intraspecific variations are *C. botryocarpa, C. diversifolia, C. espelina, C. trifoliolata, C. tubulosa* and *C. weddellii*. Of the remaining species were examined the pollen of only one specimen. There are species which pollen grains are not clearly distinguished among themselves as *C. palmata* and *C. pilosa; C. bonariensis, C. citrullifolia* and *C. ferruginea*; *C.*

alarichii, C. botryocarpa (SP 225525), *C. cabocla* and *C. villosissima; C. gracilliama* and *C. trilobata; C. rigida* and *Cayaponia* sp. (W.R. Anderson 9147); *C. botryocarpa* (INPA 147266), *C. caulobotrys, C. duckei* and *C. membranaceae.*

In conclusion, there are intra and inter-specific variations of the pollen morphological data obtained. The most important characteristics are the number and distribution of apertures, as well as the features of spines and pila. The pollen morphology offers only few parameters for taxonomic studies in *Cayaponia*, comprising itself in groups of similar species.

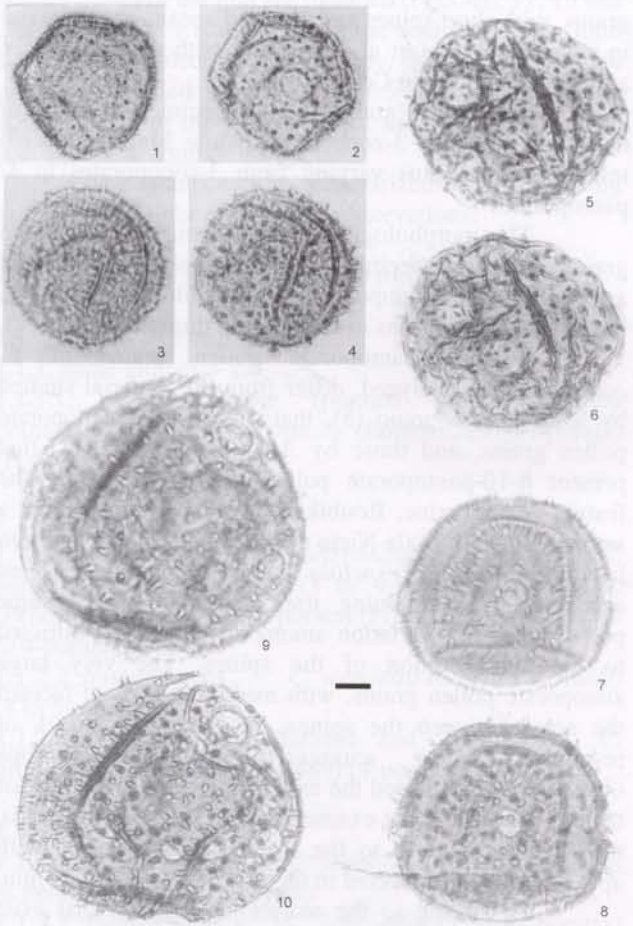

Plate I: light microscope micrographs of pollen grains of one representative species of each of the five pollen types established showing surface ornamentations and optical sections. Figs. 1 and 2, pollen type 1: *Cayaponia palmata.* Figs. 3 and 4, pollen type 2: *C. martiana.* Figs. 5 and 6, pollen type 3: *C. cabocla.* Figs. 7 and 8, pollen type 4: *C. tayuya.* Figs. 9 and 10, pollen type 5: *C. fluminensis.* (bar = 20µm)

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References

1. Ayala-Nieto, M.L., Saade, R.L. and Alvarado, J.L. (1988) Morfologia Polínica de las Cucurbitaceae de la Península de Yucatan, México. Pollen et Spores 30 (1): 5-28.

2. Barth, O.M. and Melhem, T.S. (1988) Glossário Ilustrado de Palinologia. Campinas, Ed. da Universidade de Campinas, 75 p.

3. Bates, D.M., Robinson, R.W. and Jeffrey, C. (1990) Biology and Utilization of the Cucurbitaceae. Cornell University Press Ithaca and London, 520 p.

4. Campos, S.M. (1962) Pollen grains of plants of the "Cerrado"- IV. Revista Brasileira de Biología 22 (3): 307-315.

5. Erdtman, G. (1952) Cucurbitaceae. In: Pollen Morphology and Plant Taxonomy - Angiosperms. pp. 135-137.

6. Marticorena, C. (1963) Material para uma monografia de la morfologia del pólen de Cucurbitaceae. Grana Palynologica 4 (1): 78-91.

7. Punt, W., Blackmore, S., Nilsson, S. and Le Thomas, A. (1994) Glossary of pollen and spore terminology. Utrecht, LPP Foundation-III. 71 p. (LPP contributions series n° 1)

8. Roubik, D.W. and Moreno, P.J. E. (1991) Pollen and Spores of Barro Colorado Island. Cucurbitaceae. Monographs in Systematic Botany from Missouri Botanical Garden 36: 83-84.

9. Salgado-Labouriau, M.L. (1973) Contribuição à Palinologia dos Cerrados. Anais da Academia Brasileira de Ciências, Rio de Janeiro, 281 p.

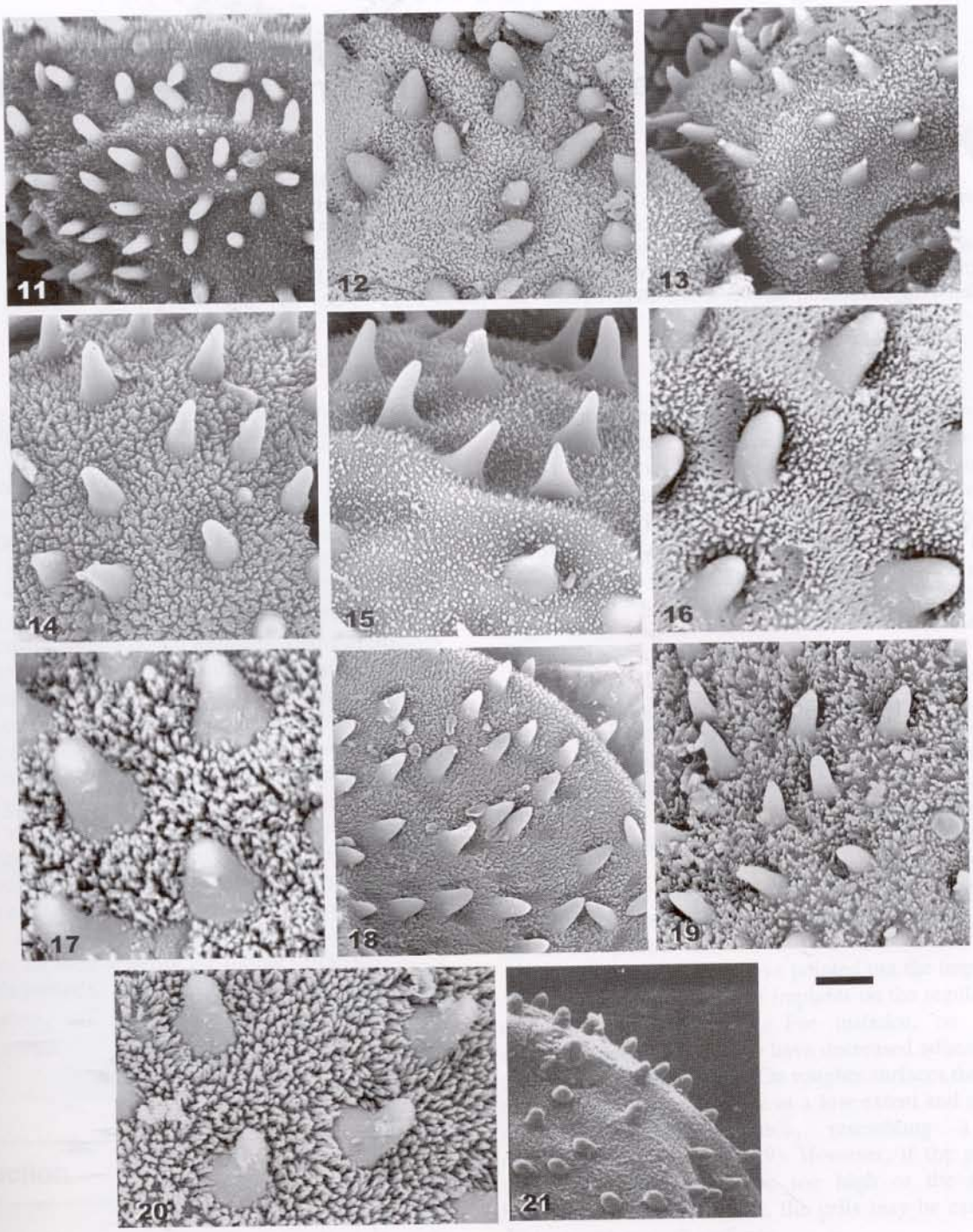

Plate II: scanning electron micrographs of the surface ornamentation of one representative species of each of the five pollen types and 9 subtypes established. Fig. 11, pollen type 1, short spines: *C. longifolia*. Fig. 12, pollen type 1, medium spines: *C. nitida* (Silva & Emmerich 3489). Fig. 13, pollen type 1, long spines: *C. nitida* (Jardim 229). Fig. 14, pollen type 2, short spines: *C. martiana*. Fig. 15, pollen type 3, short spines: *C. membranacea*. Fig. 16, pollen type 3, medium spines: *C. tubulosa* (Kuhlmann 859). Fig. 17, pollen type 3, long spines: *C. citrullifolia* (Falcão 5120). Fig. 18, pollen type 4, short spines: *C. espelina* (Gomes-Klein 2640). Fig. 19, pollen type 4, medium spines: *C. espelina* (Krieger 19446). Fig. 20, pollen type 4, long spines: *C. tayuya* (Gomes-Klein 3422). Fig. 21, pollen type 5, medium spines: *C. fluminensis*. (bar = 5µm , except Fig. 21: bar = 20µm).